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BMP9 via activate p38MAPK pathway in osteosarcoma cells, which may help us develop efficacious differentiation therapies for osteosarcoma.
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THERMALLY RESPONSIVE NANOSPHERES WITH DUAL DRUG RELEASE PROFILES FOR COMBINED CRYOTHERAPY OF OSTEOARTHRITIS

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Introduction: In this study, diclofenac (DCF) and kartogenin (KGN) were chosen as the combined osteoarthritis (OA) cryotherapy to induce anti-inflammatory activity and cartilage regeneration. Here, we designed a dual drug delivery system with thermo-responsiveness for combined therapy of OA which is composed of pluronic F127 (F127)-chitosan oligosaccharide (COS)-KGN conjugated nanospheres (F127-COS-KGN NPs) encapsulating DCF. The aims of this study were to: (1) characterize the F127/COS/KGN_{DCF} for controlled dual release by temperature change and F127-COS-KGN_{DCF}; and (2) evaluate the combined therapeutic effects of the F127-COS-KGN_{DCF} *in vitro*.
Subjects and Methods: (1) Preparation of F127-COS-KGN NPs loading DCF. The F127/COS/KGN were made by emulsification/solvent evaporation method. Conjugation of F127-COOH and KGN with COS was carried out by EDC/NHS catalysis during the NPs synthesis process. DCF was encapsulated inside the NPs by change of wall-permeability according to temperature control. (2) Controlled dual release by temperature change. The amounts of KGN and DCF released from the NPs were determined by HPLC chromatography. (3) *In vitro* chondrogenic differentiation. The hBMSC (2.5×10^5 cells, passage 3-5) were made by pellets. (4) *In vitro* anti-inflammatory activity. After induction of inflammation with lipopolysaccharide (LPS), the F127/COS/KGN_{DCF} was used to treat the cells. (5) *In vivo* thermo-responsiveness & retention time in OA joint. OA was induced surgically using ACLT and DMM in rats. After IA injection of the fluorescence dye-labelled F127/COS/KGN_{DCF}, cold temperature (5°C) were applied around the joint for 10 minutes with a cryotherapy device. Fluorescence spectrum was scanned using an IVIS-spectrum measurement system. (6) *In vivo* cyclooxygenase inhibition. Serum and synovium were collected in OA rats after IA injection of the F127/COS/KGN_{DCF}. COX-2 inhibition after cold temperature treatment was evaluated by RT-qPCR and ELISA. (7) *In vivo* cartilage regeneration. The OA rats were treated with F127/COS/KGN_{DCF} by IA injection at weeks 6 and 9 after OA induction. The distal femora in each group were dissected at 14 weeks after OA induction and evaluated by Safranin-O staining and OARSI scoring. Immunohistochemistry of COL2 and ACAN was also carried out.
Results: (1) Preparation of F127-COS-KGN NCs loading DCF. The F127/COS/KGN are ~300 nm at 37°C and expand to ~650 nm when cooled to 4°C. (2) *In vitro* release study. While the encapsulated DCF showed burst release for 6 hours after cold shock, the conjugated KGN showed sustained release for 14 days even though the temperature changed. (3) *In vitro* chondrogenic differentiation. The gene expression of COL2A1 and ACAN increased in hBMSCs pellets exposed to unconjugated KGN and both F127/COS/KGNDCF for 21 days compared with those of untreated hBMSCs. (4) *In vitro* anti-inflammatory activity. After cold shock treatment, the F127/COS/KGNDCF treated chondrocytes showed rapid decrease of IL-6 secretion. (5) *In vivo* thermo-responsiveness & retention time in OA joint. The fluorescence signals from F127/COS/KGNDCF were observed in the knee joint of OA rats up to 21 days. In particular, F127/COS/KGNDCF treated rats after cold temperature treatment showed significantly higher fluorescence intensity than those of rats untreated with cold temperature on days 2 ($p < 0.01$) and 5 ($p < 0.05$). (6) *In vivo* cyclooxygenase inhibition. After cold temperature treatment, the F127/COS/KGNDCF injected rats showed decrease of COX-2 activity.
Discussion and Conclusion: Both KGN and DCF were released independently from the F127/COS/KGN_{DCF} by temperature control. COX-2 inhibition by DCF released from the NCs after cold temperature treatment was confirmed. The F127/COS/KGN_{DCF} can be effectively combined therapeutic for OA by thermally controlled dual drug delivery.
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CORRELATION ANALYSIS OF DXA AND COMBINED USE OF QUS AND OSTA

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Background: At present, the population aging situation in China has become more and more serious. Osteoporosis is one of the deadliest diseases that affect the health of the elderly. Early detection and early prevention of osteoporosis can help to avoid serious complications of osteoporosis, such as limb brittle fractures or vertebral compression fractures. The extended life expectancy and increasing number of elderly in the population means the arrival of an aging society. Quantitative ultrasound (QUS) is a non-invasive method for evaluating bone mass density developed in the 90s. It not only reflects the bone density, but also contributes to show the bone strength and bone structure characteristics; therefore, it has the value of diagnosing osteoporosis and predicting potential fracture risks as well. At the same time it is convenient to carry and easy to operate. Asian osteoporosis self-assessment tool (OSTA) is an easy and effective way to evaluate Asian people's osteoporosis. Neither OSTA nor QUS can solely achieve the desired sensitivity and specificity when screening for osteoporosis, but it is a feasible way to combine both methods. This study aims to explore the use of combining QUS and OSTA to evaluate the risk of osteoporosis in a community of postmenopausal women.
Methods: From September 2014 to December 2014, bone mineral density of 118 postmenopausal women was measured in Guangzhou communities by quantitative ultrasound measurement and relative information such as their ages and BMI were collected through questionnaires. Patients also went through lumbar and dual-energy X-ray scans. DXA test results were taken as the gold standard of osteoporosis diagnosis, by drawing an ROC curve, this research evaluates the feasibility of the joined use of QUS and OSTA score in osteoporosis screening and determine the appropriate diagnosis point.
Results: When combined use of OSTA and for screening, the regression curve was fitted as $Y=-1.688*QUS-0.186*OSTA-3.973$. Y was considered to be a predicted value. Meanwhile, the AUC of ROC drawn by predicted value and DXA screening result is 0.847, $SE=0.041$.
Discussions and Conclusions: Quantitative Ultrasound (QUS) and OSTA score is a simple and economic method of predicting the incidence of osteoporosis in the elderly. By setting the QUS and OSTA threshold, it can effectively screen osteoporosis in patients at high risk.
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ESTABLISHMENT OF OSTEOPOROSIS MODEL IN C57/BL6 MICE BY OVARIECTOMY

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Background: To investigate the optimum timing and how long to build the osteoporosis model in C57/BL6 mice by ovariectomy (OVX).
Methods: Fifty six-week-old female C57/BL6 mice were divided into ten groups (A-J). Group A and F underwent BMD measurement by DEXA on cranium at eight-weeks-old and twelve-weeks-old, respectively. The BMD analysis of group B-E was performed at 8 weeks, 10 weeks, 12week, and 14 weeks after the mice underwent OVX at eight weeks old. The BMD analysis of group G-J was performed at 6 weeks, 8 weeks, 10 week, and 12 weeks after the mice underwent OVX at twelve weeks old.
Results: The mean BMD on the cranium of twelve-week-old mice ($0.131\pm0.030 g/cm^2$) was significantly higher than the BMD of eight-week-old mice ($0.113\pm0.042 g/cm^2$) ($P<0.05$). There was no significant difference between groups A-E. The mean BMD on the cranium of group F ($0.131\pm0.030 g/cm^2$) was significantly higher than the BMD of group H ($0.113\pm0.014 g/cm^2$) ($P<0.05$). The BMD decreased smoothly from H-J ($P>0.05$).
Discussions and Conclusions: The optimum age to build up the osteoporosis model in C57/BL6 mice is twelve weeks old and we should wait at least 8 weeks before the model is established.
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STIM1 REGULATED OSTEOBLAST DIFFERENTIATION IN THE DEVELOPMENT OF POSTMENOPAUSAL OSTEOPOROSIS

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Introduction: Calcium is required for a number of functions in the body. Past research has established the strong correlation between calcium homeostasis and supplementation leading to enhanced bone health in postmenopausal women. However, calcium supplementation is not without controversy and benefits on skeletal health need to be balanced against potential risks. Results of recent clinical trials indicate that calcium supplementation does not significantly reduce fracture risk in postmenopausal woman. The underlying mechanisms of this controversy have not been well defined. Stromal interaction molecule-1(STIM1) is localised in the endoplasmic reticulum (ER), senses $[Ca^{2+}]_{ER}$, and activates the Ca^{2+} release activated Ca^{2+} (CRAC) channel upon store depletion. Recent research found that CRAC channel plays an essential role in differentiation and function of osteoclasts and osteoblasts. Here we have investigated the role of STIM1 in the osteogenic differentiation of osteoblasts and postmenopausal osteoporosis.
Subjects and methods: BMMSCs were cultured in the presence of osteogenic induction for 14 days after being obtained from postmenopausal osteoporosis patients. We analysed the Ca^{2+} concentration and CRAC channel by Fluo-3 staining before and after osteogenic induction, respectively. STIM1 expression and osteogenic differentiation was further evaluated using quantitative real time PCR to compare expression of STIM1 and osteogenic markers. We conducted knockdown of one component of the CRAC channel, STIM1 in MC3T3-E1 using stable shRNA interference. Detection of the osteogenic gene markers, ALP activity, and